

18. Biologic Adaptations to Mineralized (Zn, Cd, Cu, Se, As) Conditions

METAL RESISTANCE IN EARTHWORMS: GENETIC ADAPTATION OR PHYSIOLOGICAL ACCLIMATION

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Pedobiologia, Vol 43 No 6, p 594-601, 1999

We hypothesised that long-term earthworm colonizers of heavily polluted metalliferous soils in the vicinity of abandoned Pb/Zn-mines have responded genetically to the selection pressures imposed upon them by the edaphic contaminants such that they have evolved into locally differentiated, metal-adapted, ecotypic populations. The hypothesis was tested by measuring and comparing the growth rates up to 36 weeks post-hatching of the F1 generation offspring of adult *Lumbricus rubellus* collected from four distinct sites: a clean calcareous reference site; a clean acidic reference site; a calcareous mine site; an acidic mine site. Four subsets of each of the four F1 populations separated, with one subset grown on food-supplemented soil of parental origin, and one subset allocated to each of the other 'experimental' field-collected soils. Growth did not yield compelling evidence of metal adaptation in either of the two mine-derived F1s. Different organism groups exploit different life-history strategies to cope with environmental stress. This tenet justifies our intention to extend our observations on F1 s beyond 36 weeks to encompass reproduction endpoints. Two further findings were noteworthy: (a) soil pH is a major determinant of juvenile earthworm growth; (b) plotting the growth data for individual worms representing the F1 offspring of parents inhabiting the acidic mine soil revealed two distinct phenotype clusters, one with a significantly faster growth rate than the other.

BIOTIC AND ABIOTIC CHARACTERISTICS OF ECOSYSTEMS ON ACID METALLIFEROUS MINE TAILINGS NEAR SUDBURY, ONTARIO

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Canadian Journal of Botany, Vol 77 No 3, p 410-425, 1 Mar 1999

INCO Ltd., a large mining company near Sudbury, Ont., deposits vast amounts of tailings on its property. These tailings contain elevated levels of Cu, Ni, and sulphides, and to curtail dust and acid drainage, INCO has undertaken a long-term project to cover its tailings with vegetation. Yearly amelioration of fresh tailings with limestone and fertilizer, followed by seeding with various grasses and herbs, transplanted conifers, and the later colonization of volunteer species of plants from adjoining forests, has resulted in the formation of ecosystems of varying age and complexity. To assess the long-term effectiveness of INCO's vegetation techniques, we studied floral diversity, attributes of developing soils, and the accumulation of Cu and Ni in various plant species growing on tailings at different stages of development. Habitat disturbance by tailings deposition, and its subsequent floral recolonization, is an example of "anthropogenic succession." Tailings that do not receive amelioration in the form of lime, fertilizer, and seeding remain free of vegetation, other than sparse clumps of the metal-tolerant grass *Deschampsia caespitosa* (L.) Beauv. Once amelioration begins, various species of grasses and herbs are able to subsist and within 8 years volunteer species begin to colonize. Substrate pH of dried tailings is less than 4 while the pH in upper horizons ranges from 5 to 6, increasing as the sites become more florally diverse; however, pH at lower depths remains less than 4. Substrate organic content in upper horizons at restored sites ranges from 4.5 to 5.0%. Water-soluble concentrations of Cu and Ni in upper horizons ranges from 1 to 5 dry mass, and 0.5 to 6 dry mass, respectively, and both metals decrease with increasing

floral diversity. Levels of Ni increase at lower depths indicating greater substrate mobility. Levels of Cu and Ni are higher in plants from tailings than in those from control sites; however, concentrations do not decrease in plants from older and more florally diverse tailings sites. Root tissues contain significantly higher concentrations of Cu and Ni than aerial tissues. Monitoring air with moss plates indicates that dispersal of airborne metallic dust remains a problem.

COPPER TOLERANCE TESTING ON *MIMULUS CUPREUS DOMBR.*, *SCROPHULARIACEAE*, IN POPULATIONS EXPOSED AND NON EXPOSED TO COPPER MINE POLLUTION

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International Conference on Heavy Metals in the Environment, 6-10 August 2000, Ann Arbor, MI
Elsevier Science Publishers, Oxford, UK

Several plant species have evolved metal tolerance as a response of increasing metal concentration in soils either by natural causes or man-polluting activities. A well described example is the evolution of a tolerant ecotype of the monkey flower (*Mimulus guttatus*) on Copperopolis, an abandoned copper mine in California. Although copper mine activities have been intense in some areas of the Andes Range in central Chile, presence of metal tolerant ecotypes has not been investigated for any plant species. Therefore, two populations of *Mimulus cupreus* from a slightly perturbed basin (Río Cipreses) and a highly copper mine perturbed one (e.g., El Teniente belonging to CODELCO-El Teniente copper mine) were tested to determine their copper tolerance. To view papers/abstracts from the conference:
<http://www.sph.umich.edu/eih/heavymetals/TechnicalProgram.html>

GROWING SNAILS USED AS SENTINELS TO EVALUATE TERRESTRIAL ENVIRONMENTAL CONTAMINATION BY TRACE ELEMENTS

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Chemosphere, Vol 40 No 3, p 275-284, Feb 2000

Two-month-old garden snails *Helix aspersa* were evaluated as sentinels of terrestrial contamination by trace elements. The snails were kept in bottomless cages for four months at four different sites in France with different pollution loads, and effects on mortality, growth, and metal bioaccumulation were monitored. Results showed that the snails efficiently bioaccumulated zinc, lead, and cadmium, occurring more in the viscera than in the foot. Snail mortality was very high at the urban site, which indicated that the laboratory-reared snails were not adapted to polluted environments like those occurring naturally in gardens and yards. Growth was also an indicator of contamination, with animals in more polluted environments exhibiting a slower growth rate.

EFFICIENT SHEDDING OF ACCUMULATED METALS DURING METAMORPHOSIS IN METAL-ADAPTED POPULATIONS OF THE MIDGE *CHIRONOMUS RIPARIUS*

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Environmental Toxicology and Chemistry, Vol 18 No 6, p 1225-1231, Jun 1999

In the River Dommel, which flows from Belgium to the Netherlands, metal accumulation was determined in larvae of midges *Chironomus riparius*, along with its loss during metamorphosis. Both reference and metal-adapted midge populations were studied. The adapted populations were collected downstream from a factory that had produced zinc and cadmium from ores. Results showed that large

differences were measured in Cd body burdens between larvae from the reference and polluted sites, but the elevated levels dropped to background body burdens in adult midges. Cadmium adaptation in the affected populations was attributed to both a higher metal excretion capacity in larvae and to a highly efficient capacity to shed accumulated Cd during metamorphosis. This was not observed for Zn, which suggested that the shedding capacity in the larvae was used first to eliminate the excess of the nonessential and potentially toxic Cd to background levels.

BIOLOGICAL ABATEMENT OF ACID MINE DRAINAGE: THE ROLE OF ACIDOPHILIC PROTOZOA AND OTHER INDIGENOUS MICROFLORA

Johnson, D.B.

Acidic Mining Lakes: Acid Mine Drainage, Limnology, and Reclamation
Springer, New York. ISBN: 354063486X. p 285-302, c1998

ARSENIC UPTAKE FROM CONTAMINATED SOILS BY A HYPERACCUMULATING FERN

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International Conference on Heavy Metals in the Environment, 6-10 August 2000, Ann Arbor, MI
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There is a great need for reliable and cost-effective technologies capable of reducing arsenic in soils to environmentally acceptable levels. Phytoremediation, a plant-based technology, has been successfully used to remove heavy metals from contaminated soils. However, no plant capable of hyperaccumulating arsenic was recognized until recently. The researchers discovered an extremely efficient arsenic-hyperaccumulating fern. Fern samples were collected from an arsenic-contaminated soil and analyzed for arsenic concentrations. In addition, ferns after growing in artificially contaminated soils for up to eight weeks in a greenhouse were harvested and analyzed for arsenic concentrations. The highest arsenic concentrations in the aboveground biomass in ferns growing in the arsenic contaminated soil in the field was 7,500 ppm, with arsenic concentration in aboveground biomass being up to 200 times greater than those of soils. After four weeks, the arsenic concentration reached over 2% in the aboveground biomass of the fern growing in the soil spiked with 500 ppm arsenic. Obviously, this fern has an extraordinary capability to uptake a large quantity of arsenic from soils and translocate them to aboveground biomass. The fern has a great potential to be used for phytoremediating arsenic-contaminated soils and wastes. To view papers/abstracts from the conference:
<http://www.sph.umich.edu/eih/heavymetals/TechnicalProgram.html>

COLONIZATION AND DEVELOPMENT OF VEGETATION IN MINING LAKES OF THE LUSATIAN LIGNITE AREA IN DEPENDENCE ON WATER GENESIS

Pietsch, W.

Acidic Mining Lakes: Acid Mine Drainage, Limnology, and Reclamation
Springer, New York. ISBN: 354063486X. p 169-196, c1998

ALCALIGENES EUTROPHUS AS A BACTERIAL CHROMATE SENSOR

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Applied Environmental Microbiology, Vol 64 No 2, p 453-458, Feb 1998

The bacterium, *Alcaligenes eutrophus* CH34, has been adapted to survive in environments with high concentrations of toxic metals. In the presence of chromium, the mechanism of chromate resistance involves reduced accumulation. Results are presented from a study that evaluated the regulation of the *chr* and *cnr* genes using *lacZ* fusions in order to develop a metal-sensing bacterial strain. Of all the fusions in the *chr*-*cnr* region tested, fusions in *chrA* were the most specific and gave the strongest responses. The main inducers were chromate and dichromate. The data suggested a connection between a chromosomally encoded sulfate reduction pathway and the plasmid-borne *chr* chromate resistance system.

INCREASED CADMIUM EXCRETION IN METAL-ADAPTED POPULATIONS OF THE MIDGE CHIRONOMUS RIPARIUS (DIPTERA)

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Environmental Toxicology and Chemistry, Vol 15 No 3, p 332-339, Mar 1996

Laboratory-reared offspring of cadmium-adapted and -nonadapted midges *Chironomus riparius* were used to conduct Cd accumulation and elimination experiments. A first-order one-compartment model was fitted to the experimental data to obtain estimates for accumulation and elimination. The experimental protocol is described. Larval growth ceased completely during accumulation experiments, but steady growth was observed during elimination experiments. Differences in pupation rates were in accordance with larval growth patterns. First-order one-compartment models did not always provide a good description of the experimental data. The results demonstrated significant differences in elimination rates between the populations because guts of the two populations were characterized by an increased excretion efficiency in the Cd-adapted group.

RESISTANCE OF EISENIA FETIDA (OLIGOCHAETA) TO CADMIUM AFTER LONG-TERM EXPOSURE

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Ecotoxicology and Environmental Safety, Vol 4 No 2/1, p 75-80, 1999

The reliance on earthworms as test organisms in risk assessment studies of polluted environments raises the question whether they can evolve resistance, e.g., by adaptation to specific toxicants. Protection criteria may be biased if sensitivity data from adapted populations are used. Increased resistance to the heavy metal cadmium has not yet been determined for terrestrial Oligochaeta. *Eisenia fetida* was exposed to a sublethal concentration of cadmium sulfate for more than 10 generations. Clitellate worms from this culture were used in experiments to determine the extent of possible tolerance for the heavy metal. Preexposed animals as well as worms with no previous history of exposure to cadmium were exposed to a control substrate without cadmium and also to two substrates with 600 and 1200 µg g⁻¹ SUP - SUP 1 cadmium. Changes in biomass, cocoon production, and hatching success were monitored. The results obtained indicated that in both substrates in which cadmium was present the preexposed worms performed better than the unexposed worms with respect to growth rate but not reproductively. In the substrate without cadmium the preexposed worms exhibited signs of poisoning after a few weeks. Preexposed and unexposed worms were also exposed to concentrations of 1500 to 4000 µg g⁻¹ cadmium sulfate in an artificial medium for a period of 2 weeks. The preexposed worms survived higher concentrations of cadmium than the unexposed group and some specimens from the unexposed group had a gross increase in body

fluids. It is concluded that worms with a long-term history of exposure to the metal developed resistance to cadmium.

EFFECTS OF MYCORRHIZAE AND OTHER SOIL MICROBES ON REVEGETATION OF HEAVY METAL CONTAMINATED MINE SPOIL

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Environmental Pollution, Vol 86 No 2, p 181-188, 1994

Andropogon gerardii is highly dependent on mycorrhizal fungi in native prairie, while *Festuca arundinacea* is a facultative mycotroph and relies on mycorrhizal symbiosis only in extremely infertile soils. Regardless of microbial amendments, neither plant species was able to establish and grow in the mine tailings. Although *F. arundinacea* was more highly colonized by mycorrhizal fungi than *A. gerardii*, neither microbial amendment affected growth of fescue in any soil. In several treatments mycorrhizal fungi adapted to uncontaminated soil stimulated plant growth more than mycorrhizae adapted to the moderately contaminated soil, but mycorrhizal fungi adapted to contaminated soil did not increase productivity of plant growth in contaminated soil more than fungi adapted to uncontaminated soil. *A. gerardii* plants inoculated with mycorrhizal fungi retained more Zn in roots than in shoots. Mycorrhizae did not affect translocation patterns in *F. arundinacea*, suggesting that the mycorrhizal dependence of a plant species is correlated with the retention of metals in roots.

LIFE-HISTORY PATTERNS IN REFERENCE AND METAL-EXPOSED EARTHWORM POPULATIONS

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Ecotoxicology, Vol 8 No 2, p 133-141, 1999

The growth and development of F1 *Lumbricus rubellus* bred from reference and one metal-exposed (smelter) populations were assessed in soils from both sites. In the clean soil, faster growth (as reflected by mean weight) and maturation were found at selected time intervals for the smelter worms. This result is in agreement with predictions from life-cycle models, which indicate that polluted-site populations will be adapted for faster growth, earlier maturation and increased reproductive effort. Life-history adaptation is not however the only explanation for the differences in maturation rates found. During the exposures, mortality of smelter worms was higher than for the reference strain. This resulted in a reduction in the density of the smelter strain. In earthworms, both growth rate and maturation time are known to be density dependent. Thus it is probable that density-mediated responses of growth and development, rather than adaptation, are primarily responsible for the observed life-history. Exposure of the two strains to the smelter site soil was carried out to quantify differences in performance indicative of physiological resistance in the smelter worms. In fact, no consistent differences in growth or maturation were found. Thus it is unlikely that growth and development responses of *Lumbricus rubellus* are adapted to the metals present in soils at the smelter site.

FACTORS EXPLAINING THE DISTRIBUTION AND SITE DENSITIES OF THE NEOSHO MADTOM (NOTURUS PLACIDUS) IN THE SPRING RIVER, MISSOURI

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U.S. Geological Survey Toxic Substances Hydrology Program: Proceedings of the Technical Meeting, 8-12 March 1999, Charleston, South Carolina. Volume 1, Contamination from Hard-Rock Mining

U.S. Geological Survey Water-Resources Investigation Report 99-4018A, Vol 1, p 269-279, 1999

The Neosho madtom, a Federally-listed threatened species endemic to the Arkansas River system, is presently restricted to selected mainstem reaches of the Neosho, Cottonwood, and Spring rivers in Missouri, Kansas, and Oklahoma. These rivers are affected by anthropogenic factors such as municipal waste discharges and agricultural runoff. The Spring River also drains the Tri-State Mining District, where zinc-lead mining occurred in the past. Our objective was to assess effects of water quality degradation, due mainly to mining-related contaminants, on aquatic communities of the Spring River by comparison with those of the Neosho-Cottonwood system. We found higher densities of *N. placidus*, finer-textured riffle substrate, and lower concentrations of cadmium and lead in benthic macro-invertebrates in the Neosho-Cottonwood system than in the Spring River. In the Spring River, we found no substrate differences between sites with and without *N. placidus*; however, taxonomic richness of the benthic macro-invertebrate and fish communities were greater, densities of *N. placidus* and other fishes were higher, and concentrations of zinc and cadmium in benthic macro-invertebrates were lower at sites with *N. placidus*. Pore waters from three sites in the Spring River system were toxic to *Ceriodaphnia dubia*; mortality was greater than 80%, and there was no reproduction. Concentrations of zinc and cadmium in pore waters and sediment were high at these sites relative to non-toxic sites, and had SEM/AVS ratios considered potentially toxic. Toxicity tests, concentrations of metals in benthic macro-invertebrates, toxic unit (UT) modeling of pore waters, and an empirical habitat model support a hypothesis of contaminant involvement in the distribution of *N. placidus*. The paper is available at <http://toxics.usgs.gov/pubs/wri99-4018/Volume1/index.html>

NICKEL TOLERANCE OF PHYTOPLANKTON ISOLATED FROM A RECOVERING LAKE NEAR SUDBURY CANADA

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International Conference on Heavy Metals in the Environment, 6-10 August 2000, Ann Arbor, MI
Elsevier Science Publishers, Oxford, UK

The current study compared “free” metal tolerance to Ni of three species of phytoplankton, *Urosolenia eriensis*, *Cosmarium minimum* and *Senedesmus acutus* isolated from Alice Lake, Sudbury, Canada in an attempt to determine if Ni tolerance was related to presence of these species in the lake. Alice Lake was subjected to 60 years of Ni and Cu pollution from both aerial inputs and runoff from slag piles from a nearby Ni-Cu smelter. After the closure of the smelter and building of the super stack in 1972 the air quality improved immediately and water quality began to improve by the late 1970s and early 1980s. Phytoplankton, which had been all but absent from the lake, had recovered by the mid 1980s. Tolerance was measured in defined media allowing for the modeling of speciation, “free” Ni, using GEOCHEM-PC (ver. 2.) Nickel tolerance (Effective Concentration causing a 50% reduction in growth = EC50) in a defined medium was compared with ambient lake water concentrations and the presence of each species from the mid 1980s to the mid 1990s. Results from these comparisons suggest that there are different mechanisms of tolerance, unique to each species. To view papers/abstracts from the conference: <http://www.sph.umich.edu/eih/heavymetals/TechnicalProgram.html>